AD-A239 338

UMENTATION PAGE

Form Approved
OMB No 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	May 1991	THESIS/DISSERTATION

4. TITLE AND SUBTITLE
Experimental Model for the Study of Periodontal Wound Healing

5. FUNDING NUMBERS

6. AUTHOR(S)
Robert R. Burnett, Major

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
AFIT Student Attending: University of Texas

8. PERFORMING ORGANIZATION REPORT NUMBER
AFIT/CI/CIA- 91-061

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
AFIT/CI
Wright-Patterson AFB OH 45433-6583

10. SPONSORING MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release IAW 190-1
Distributed Unlimited
ERNEST A. HAYGOOD, 1st Lt, USAF
Executive Officer

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

14. SUBJECT TERMS

15. NUMBER OF PAGES
72

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT

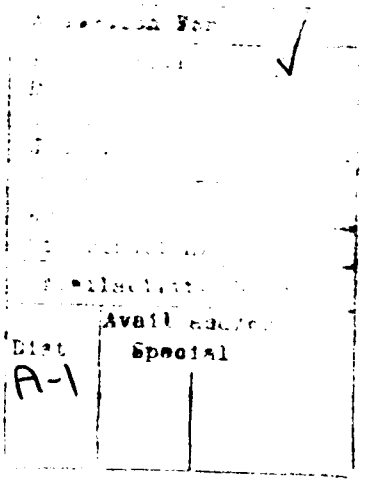
EXPERIMENTAL MODEL FOR THE STUDY OF PERIODONTAL WOUND HEALING

A
THESIS

Presented to the faculty of
The University of Texas Graduate School of Biomedical Sciences
at San Antonio
in Partial Fulfillment
of the Requirements
for the Degree of
MASTER OF SCIENCE

By

Robert Ray Burnett, D.D.S.



San Antonio, Texas

May 1991

EXPERIMENTAL MODEL FOR THE STUDY OF PERIODONTAL WOUND HEALING

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April 1, 1991
Date

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Dean

DEDICATION

This thesis is dedicated to all of my loved ones that sacrificed as much as anyone in achieving the completion of this research. First, to my understanding and loving bride, Christy, thank you for "putting up" with the restrictions on our time together. God willing, the future will hold additional opportunities for adventure and sharing our lives together. And to my parents and sister, thank you for providing the positive influence on my life that gave me not only the opportunity to participate in projects such as this, but also the drive for achievement.

ACKNOWLEDGEMENTS

I would like to thank all of the individuals that contributed and guided my efforts on this project. My mentor Dr. Kornman, committee member advisors Dr. Hallmon, Dr. Waldrop and Dr. Aufdemorte, technical advisors Dr. Rylander, Dr. Lundgren and Dr. Swan and assistants Lisa Lara and Sonia Bustamante, each played a viable role in bringing this project to fruition. I have an appreciation and respectful attitude for the massive committment necessary in achieving a research project. I hope I can repay a portion of my indebtedness by contributing to future research and aspiring researchers.

EXPERIMENTAL MODEL FOR THE STUDY OF PERIODONTAL WOUND HEALING

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The University of Texas Graduate School of Biomedical Sciences
at San Antonio

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In order to evaluate the influence of biologic mediators on the regenerative capabilities of the periodontium, it is essential to establish a baseline for wound healing events in the absence of disease. The primary goal of this pilot study was to evaluate a fenestration wound healing model. Surgically created defects were made into the dentin through alveolar bone fenestrations at the mid-root level in 8 sites per arch in 4 primates (*Macaca mulatta*). Initially, surgery was performed in one arch per animal. A total of 32 fenestration wound sites were made. The following treament modalities were accomplished in order to further define the fenestration wound healing model. (1) Eleven (34%) sites received a titanium screw within the root so the screw head was even with the surrounding root surface. (2) Eleven (34%) sites received the same treatment as in (1) but in addition received a polytetrafluoroethylene membrane bridging over the fenestration. (3) Five (16%) sites received a root surface preparation to remove periodontal ligament, cementum and some dentin (controls). (4) Five (16%) sites received the same treatment as in (3) but in addition received a polytetrafluoroethylene membrane bridging over the fenestration. Fifteen weeks later,

32 fenestration wound sites were created in the opposing arches. These included: (1) Eight (25%) sites with titanium screws and a collagen membrane bridging over the fenestration. (2) Eight (25%) sites with titanium screws and a collagen membrane incorporated with Transforming Growth Factor-ß1. (3) Eight (25%) sites with a root surface preparation to remove periodontal ligament, cementum and some dentin. These sites were covered with a collagen membrane. (4) Eight (25%) sites with the same treatment as (3) but with a collagen membrane incorporated with Transforming Growth Factor-ß1. The animals were sacrificed five weeks after the second surgery (twenty weeks after the initial surgery). The following histological observations were made: (1) twenty week titanium sections with and without a polytetrafluoroethylene membrane had cementum-like material, "cementointegration", in intimate contact with the surface of the screws; (2) twenty week non-titanium sections with and without polytetrafluoroethylene membranes demonstrated complete healing with a perpendicular-oriented periodontal ligament, cementum and bone covering the defects; (3) a foreign body giant cell reaction was present adjacent to the polytetrafluoroethylene membrane; (4) five week non-titanium sections demonstrated a parallel-oriented periodontal ligament, cementum and bone covering the defects with no apparent differences at this time period between the use of collagen membranes with or without Transforming Growth Factor-ß1; (5) collagen membranes with and without Transforming Growth Factor-ß1 were biocompatible in this animal model, manifesting an indistinguishable demarcation between the membrane and new bone formation at five weeks; (6) five week titanium sections had no cementum formation on the titanium screws, however new cementum was present on the adjacent surgically denuded dentin; no histological differences were seen between titanium sites with or without Transforming Growth Factor-ß1.

These results suggest that the fenestration wound model in a non-human primate has a high propensity for healing and thus may serve as a valuable model for the

assessment of wound healing events. The observation of healing events in the absence of disease provides a predictable model for evaluating optimal effects of various procedures on the healing of periodontium. This model naturally excludes oral epithelium, microbial and hygiene influences and anatomical variables, in addition to maintaining a functional dentition. The lack of dissimilarities in healing with and without exclusion membranes suggests this fenestration wound may be of inadequate size to demonstrate differences in healing during prolonged evaluation times. Larger size defects and/or earlier time periods should be evaluated to determine the influence of potential periodontal treatment modalities on wound healing and to characterize the early events leading to regeneration of the periodontium.

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I. INTRODUCTION

Periodontal disease is the major cause of tooth loss after the age of 35 (Bureau of Economic and Behavioral Research, 1982). In 1985, the National Institute of Dental Research reported that nearly 77% of employed persons age 18-64 and over 95% of persons 65 years of age and older demonstrated periodontal attachment loss. In the senior population, 41% were totally edentulous and only 2% had complete dentitions (excluding third molars). Periodontal treatment needs command a significant percentage of health care provider time and health care dollars (Douglass *et al.*, 1984, 1985). Periodontal disease is characteristically associated with destruction of the periodontium including attachment loss and possible ultimate loss of the tooth (Loe *et al.*, 1978, 1986; Becker *et al.*, 1979; Buckley and Crowley 1984). Therapy for these diseases should be directed toward eliminating the disease process and regenerating lost periodontium accompanying the disease process. Unfortunately, little is known of the healing potential or specific mechanisms governing the healing sequence of the wounded periodontium. Numerous periodontal therapeutic adjuncts including epithelial exclusion membranes, growth factors, antibiotics and root conditioning agents have been proposed in periodontal therapy to promote new attachment and/or regeneration of periodontium lost to periodontal disease. Most *in vivo* wound healing studies have utilized animal models with surgically created periodontal defects in an attempt to mimic attachment loss observed in humans. These models complicate the study of healing events due to a myriad of oral environmental variables. Although such models are important in evaluating wound healing, initial *in vivo* studies should attempt to minimize such variables. Periodontal wound healing in the absence of the oral environment may provide information regarding the healing sequence and the efficacy of various adjunctive materials.

An additional variable that can prohibit the comparison of wound healing studies is the diversity of animal models utilized in wound healing research. The size, age, oral

bacterial flora, dental anatomy, immune response and susceptibility to periodontal disease between and within species may effect the results of these studies.

This pilot study describes an *in vivo* closed wound healing model that will allow for the direct comparison of periodontal wound healing events effectively enclaved against the influences of the oral environment. Such a model has potential to evaluate healing events leading to regeneration of the periodontium. Results utilizing this model should provide an objective appraisal of the regenerative potential of adjunctive materials in periodontal wound healing. This pilot project evaluated wound healing associated with and without wound healing adjuncts. Wound healing adjuncts included non-resorbable membranes, resorbable membranes with and without growth factor, and titanium implant materials.

II. LITERATURE REVIEW

A. Periodontal Wound Healing Models

In order to evaluate wound healing events subsequent to various periodontal treatments, a variety of wound healing models have been utilized. The literature is replete with periodontal wound healing studies. Many have examined healing associated with periodontal treatment of naturally occurring periodontitis, while others have evaluated treatment of experimentally induced periodontitis. While such studies have contributed immensely to our understanding of wound healing processes, numerous problems have accompanied the interpretation of respective results. Various animal models including humans, non-human primates, dogs and other species have been utilized, accounting for a vast array of potential response. Although some non-human species have naturally occurring periodontitis (e.g., the beagle dog), many dissimilarities exist between this model and the human. For example, dental anatomy, oral microbiota, hygiene, diet, and immune response can vary remarkably between species. Experimentally produced periodontitis is also difficult to compare to naturally occurring periodontitis. Using a monkey model, Isidor *et al.* (1985) suggested that healing subsequent to periodontal therapy was similar in root surfaces previously exposed to periodontal disease and those having experimentally induced periodontitis. Results from other studies, utilizing a beagle dog animal model, have contradicted these results (Vande Velde *et al.*, 1989, Kon *et al.*, 1991). Experimentally induced defects that were chronically irritated with steel matrices and plaque accumulation for 3 months subsequent to creation of the surgical defects demonstrated a large percentage of spontaneous regeneration.

Some wound models have reduced the number of variables by isolating experimental sites from the external environment. This reduces or eliminates microbial and hygienic influence, some anatomical variables and provides exclusion of the epithelium. These models may promote healing by providing isolation of target cell

populations from the external environment. In addition, healing can be assessed in a normal non-diseased periodontium. The following sections review the use of subcrestal fenestration or window wound models in periodontal research.

B. Fenestration Wound Healing Event Studies

The fenestration or window wound model dates as far back as 1927 when Beckwith *et al.* (1927) and Beckwith and Williams (1928) studied the regeneration of the periodontium utilizing a model which created a fenestration through the gingiva, alveolus, periodontal ligament, cementum and dentin. This was created by passing a dental bur through the periodontal structures in incisor teeth of rabbits, guinea pigs and cats. No primary closure was attempted over the wound site, allowing potential ingress of bacteria and clinical infection in some sites. Despite the lack of primary closure, many areas healed uneventfully. Histological sections at varying intervals up to 20 weeks demonstrated regeneration of cementum, periodontal ligament and alveolar bone in the wounds.

A similar model which included primary closure of the external wound was described by Beube (1947). A semilunar incision was made in the alveolar mucosa facial to canine teeth in dogs and one human, allowing access to create a fenestration wound beneath the mucoperiosteal flap. Concavities, 6.0 mm square, were made by removing buccal alveolar bone, periodontal ligament, cementum and dentin. Flap margins were then replaced and sutured. In the dog models at 24 hours and 3.5 days, an inflammatory infiltrate was present. Fibroblasts were apparent by day 8. By day 27, new cementum had covered the exposed dentin and new bone had covered the wound site. A disoriented fibroblastic tissue separated the newly generated bone from the new cementum. By two years, new alveolar bone was as dense as adjacent bone; the periodontal ligament was structurally well arranged; and a non-uniform thickness of cementum was present over surgically exposed dentin and preexisting cementum. Interestingly, the author reports a similar operation was performed on a human tooth not

affected by periodontal disease. Formation of new cementum, periodontal ligament and bone were present six months post-wounding.

Jansen *et al.* (1955) also utilized flap access to create subcrestal fenestration wounds in dogs. Healing adjacent to these sites was compared to healing of experimentally created sulcular lesions subjected to the oral environment (e.g., microbial contamination and ingrowth of oral epithelium). These sulcular lesions were produced with a scalpel or dental burs, damaging the periodontal ligament, the alveolar crest and the root surface. The fenestration wounds were produced with burs, removing labial alveolar bone, periodontal ligament, cementum and some dentin. Histologic sections at 2 to 3 months demonstrated regeneration of cementum, periodontal ligament and bone in many but not all fenestration sites. The crestal and supracrestal wound sites demonstrated downgrowth of epithelium and occasional new cementum and periodontal ligament formation.

Melcher (1970) and Gould *et al.* (1977) examined the differentiation of individual cell populations and their role in the repopulation and healing of periodontal wounds in similar wound models but in different animal models. Melcher (1970) used an extraoral approach to expose the alveolus of mandibular first molars in albino rats. Fenestrations were created to expose the periodontal ligament in the middle one-half to two-thirds of the mesial-buccal root. The periodontal ligament and some of the underlying cementum were removed from either the apical or coronal half of the fenestration site. The flaps were replaced and sutured. The animals were sacrificed at either 4 days, 1, 2, 3, or 4 weeks. No new cementum deposition was seen, however, the periodontal ligament space was partially repopulated with cells which were thought to have derived from the periodontal ligament either remaining within the wound and/or from the periodontal ligament adjacent to the wound. Boney ankylosis was evident in wound sites devoid of periodontal ligament, while no ankylosis was observed in sites that retained or were repopulated with periodontal ligament. The author hypothesized that the periodontal

ligament may inhibit osteogenesis, thus accounting for the lack of ankylosis in the sites repopulated with periodontal ligament cells.

Utilizing a similar extraoral approach, Gould *et al.* (1977) examined the location, migration and division of progenitor cells in the periodontal ligament by utilizing Melcher's (1970) fenestration wound model in mice. Wounding was accomplished to stimulate mitotic activity of cells in the periodontium. The wound consisted of removal of 0.8 mm diameter of alveolar bone. An attempt was made to leave the periodontal ligament and underlying cementum intact. The majority of cells undergoing mitosis were associated with blood vessels and thus were identified as paravascular cells. Subsequent research (Gould *et al.*, 1980) utilized the same model but placed a piece of sterile mylar film over the alveolar wound to segregate it from the overlying connective tissue. Radioautographic studies indicated that progenitor cells were isolated to within 200 um of the margin of the wounded periodontal ligament. These cells often divided on the periphery or migrated to the wound and divided during or after migration. A population of paravascular progenitor cells in the periodontal ligament was located within 5 um of blood vessels. The number of progenitor cells remained relatively stable during healing.

Transmission electron microscopy was utilized to evaluate healing events in a similar fenestration wound model in adult rats by Knox and Aukhil (1988). An extra-oral approach was utilized to place fenestration wounds of less than 1 mm in diameter on the buccal aspect of the mesial root of the mandibular first molar. Alveolar bone, periodontal ligament and some or all cementum were removed from the site. A Nuclepore membrane (pore size 0.1 um) was placed over the site to prevent cell contamination from the mucoperiosteal flap and to encourage repopulation by adjacent periodontal ligament cells. The membrane also created a periodontal space in the wound. Transmission electron microscopy consistently revealed an electron-dense material between the denuded root surface and the regenerating cementoid matrix in 2

and 4 week specimens. The authors speculated that the latter may represent a glycoprotein or glycosaminoglycan layer necessary in the sequence of regeneration of the periodontium.

Bernimoulin *et al.* (1978) positioned dermal connective tissue grafts over mid-root facial fenestrations in dogs and replaced the flaps over these grafts prior to suturing. Control fenestration sites did not receive dermal grafts. Following 40 and 60 day healing periods, histology revealed no significant differences in healing between treatments. Bone repair, new cementum and Sharpy's fibers were often present in both experimental and control sites. The authors concluded that the connective tissue grafts had no apparent benefit in cementum regeneration, and did not adversely affect healing.

Nyman and Karring (1979) utilized a fenestration wound model somewhat different from the aforementioned to examine if new attachment was accompanied by formation of bone previously removed surgically. A semilunar flap was made at the height of convexity overlying the root apices of premolars in two Beagle dogs with otherwise healthy periodontiums, an attempt was made not to disturb cementum or the supracrestal connective tissue attachment, while 5 to 7 mm of coronal buccal alveolar bone was removed between the mesial and distal line angles of the experimental tooth. Thus the "window" created included removal of the alveolar crest at the experimental site. Flaps were replaced and sutured. The animals were sacrificed at 8 months. Light microscopy revealed that new cementum and fibrous reattachment occurred in the supra-alveolar area. Alveolar bone regeneration ranged from 0.5 mm to 3.7 mm; however, only 2 of 8 experimental sites demonstrated regeneration approaching a distance 1 mm from the apical extent of the junctional epithelium. Supra-alveolar connective tissue attachment ranged from 1.2 mm to 4.2 mm. These results led the authors to conclude that coronal extension of connective tissue attachment in regeneration is not necessarily accompanied by alveolar bone regeneration.

Nalbandian and Frank (1980) utilized electron and light microscopy to study regeneration of the periodontium in a cat fenestration wound model. Semilunar incisions through the alveolar mucosa on the facial surface of canine teeth facilitated creation of a 2 x 3 mm fenestration over the facial root surface to a depth of 0.3 to 0.5 mm within the root surface. New bone and cementum were seen at 33 days. The new cemental surface was generally thicker at the mesial and distal edges of the lesions. Although new cementum had a variable thickness, precementum had a uniform thickness of approximately 15 um. Connective tissue was generally parallel to the tooth surface, but had a tendency to be more perpendicular, suggestive of Sharpey's fibers, when cementum was present.

Similarly, Nyman *et al.* (1982) placed U-shaped incisions to reflect a mucoperiosteal flap over maxillary lateral incisors and mandibular canines in 3 adult monkeys (*Macaca cynomolgus*). Fenestrations were made over the roots to remove buccal and interdental alveolar bone, periodontal ligament and cementum extending from a level 2 mm apical to the alveolar crest to a level down half the root length (fenestration apical-incisal length range = 2.0 to 5.1 mm). Millipore filters were placed over the fenestrations and 2-3 mm beyond their borders to prevent gingival connective tissue from contacting the root surface. Flaps were replaced and sutured. The animals were sacrificed at 6 months. New cementum extended 25-100% and new bone 0-100% from the apical extent of the fenestration coronally. Fifty percent of the specimens demonstrated a connective tissue adhesion in the coronal aspect of the fenestration without evidence of new cementum or fibrous attachment. The alveolar bone coronal to the fenestration had resorbed in 6 out of the 12 specimens. The authors concluded that the periodontal ligament has the ability to form new cementum and a new connective tissue attachment on surgically denuded root surfaces.

The influence of physical barriers on progenitor cells in 4 x 4 mm fenestrations created on the facial surface of canines in dogs was examined by Aukhil *et al.* (1986). A

Nucleopore membrane was luted with cyanoacrylate resin onto the surgically exposed dentin surface within the fenestration allowing for 0.5 millimeter of exposed dentin on the periphery of the membrane. A Millipore membrane was subsequently placed over the fenestration wound and the flaps were replaced and sutured. After 3 months of healing, histological examination indicated new cementum formation only in those areas not covered by the Nucleopore membrane. The authors hypothesized that "...progenitor cells from the periodontal ligament will differentiate into cementoblasts when they come in contact with root dentin and that such differentiation can be prevented by interposing a physical barrier between the progenitor cells and the root dentin."

Iglhaut *et al.* (1988), following the reflection of mucoperiosteal flaps, created 3 x 3 mm fenestrations at mid-root level to remove buccal cortical plate, periodontal ligament and cementum in multiple teeth in four monkeys (*Macaca fasicularis*). A millipore filter (pore size 3 um) was placed over each fenestration and the flaps were replaced and sutured. Wounding and sacrifice were scheduled to provide healing periods of 1 hour, 1, 2, 3, 7 and 21 days. Tritiated thymidine was administered prior to sacrifice and autoradiographs were used to evaluate progenitor cell kinetics. Cell migration into the wounds was evident at 3 days with cells arising from both the bone and periodontal ligament. Cell migration was also seen at day 7, but very little migration was evident at 21 days. No significant difference in labeling index was seen between the bone and the periodontal ligament compartments for any of the observation periods. Thus, both the periodontal ligament and bone compartments were considered as potential suppliers of cells into the wound model. Only the immediate adjacent area of approximately 200 um demonstrated premitotic labelling. These cells may continue to undergo amplifying cell division as they migrate. Based on these findings, physical barriers do not guarantee the exclusion of all cell types other than those from the periodontal ligament, as the bone compartment may also be a potential source of cells.

C. Evaluation of Pulpal Vitality and Fenestration Wound Healing

A model similar to the fenestration model includes the apices of human teeth damaged due to inflammation or cystic lesions. When treated with root canal obturation and apicoectomy, these teeth demonstrate variable regeneration of previously lost periodontium removed via the disease and/or treatment process (Andreasen 1973). Healing over a period of 0 to 14 years varied from the reestablishment of the periodontium with cementum, periodontal ligament and bone formation; to scar tissue, granulation tissue or inflamed connective tissue with or without limited cementum formation. An association between cementum repair and the formation of functionally arranged periodontal ligament fibers was identified during healing.

Hellden (1972) studied periodontal healing in 72 human patients (88 teeth) associated with vital teeth, non-vital non-endodontically treated teeth, and endodontically treated teeth. Semilunar flaps were reflected and 3 mm diameter fenestrations were made at the mid-root level to remove alveolar bone, periodontal ligament and cementum leaving exposed dentin. The first signs of cementum formation were observed at 23 days. Cementum formation always started at the periphery of the wound. Alveolar bone formed more rapidly than cementum, maintaining the curvature of the root surface and a functional width for the developing periodontal ligament. No ankylosis was seen. The periodontal ligament was initially oriented in a parallel configuration and assumed an oblique direction after 2 to 3 months. Some sites developed a clinical infection which resulted in an ingrowth of epithelium which totally or partly prevented regeneration. No significant healing differences were seen between vital teeth, non-vital non-endodontically treated teeth or endodontically treated teeth.

D. Evaluation of Adjunctive Treatment Modalities and Their Influence on Healing in the Fenestration Wound Model

Register (1973) was the first researcher to utilize the fenestration wound model in the evaluation of various treatment materials. A labial partial thickness flap was reflected

and a 5 mm diameter fenestration, 3 mm apical to the alveolar crest allowed removal of periosteum, labial bone, periodontal ligament, cementum and some dentin in incisor teeth of two macaque monkeys and one mongrel dog. The sites were exposed to 0.6 N hydrochloric acid for 15 minutes, rinsed with sterile saline and the flaps replaced. Contralateral control fenestrations were exposed to sterile saline. When compared to controls, the monkey experimental teeth demonstrated advanced wound repair at 6 weeks and 3 months with new cementum and bon formation; one control site demonstrated similar new cementum and bone compared to acid treated wounds at 3 months. Six week histologic sections of the acid treated dog teeth demonstrated a comparably less new cementum and bone than that seen in the monkeys. Acid treated sites had a consistent cementum attachment to the demineralized dentin zone versus artifact separation seen in controls.

Nalbandian and Cote (1982) using a similar design as Register (1973), exposed the buccal alveolar plate with a semilunar incision over canine and mandibular first molars in five Beagle dogs with healthy periodontiums. A rectangular fenestration, approximately 4 x 7 mm was created over the root surface, removing bone, periodontal ligament, cementum and superficial dentin. Two identical cylindrical cavities 2 mm in diameter and 1 mm deep were made in the root at each fenestration site. The first cavity was prepared and exposed to citric acid (pH 1.0) for 4 minutes. The area was irrigated and a second control cavity was placed, followed by irrigation, flap replacement and suturing. Cavities were alternated from site to site so that the citric acid treated site would be coronal in 50% of the sites and apical in 50%. Healing periods ranged from 60 to 126 days. New cementum, periodontal ligament and bone developed in control and experimental sites. In general, new cementum was thicker and covered a greater surface area in the experimental sites. In addition the experimental sites demonstrated less artifactual separation of the cementum - dentin surface. New bone formation was extensive with only one experimental site demonstrating ankylosis.

In a subsequent study evaluating citric acid's effect on healing, Pettersson and Aukhil (1986) reflected buccal mucoperiosteal flaps in 6 Beagle dogs and created 4 x 3 mm fenestration wounds by removing buccal bone, periodontal ligament and cementum from premolar teeth. Citric acid (pH 1) was applied for 3 minutes to the exposed root surfaces on one side of the mouth while distilled water was used on the contralateral side. The sites were irrigated and millipore filters placed to prevent flap connective tissue from contacting the experimental site. Flaps were replaced and sutured. Sacrifice occurred at 3 months. New cementum and attachment were present in experimental and control sites, however, the percentage of root surface with new attachment was significantly less in experimental sites. No significant difference existed in the amount of coronal and apical regeneration of new attachment. Resorption and ankylosis were seen more often in experimental sites. The authors suggest that citric acid may have damaged adjacent periodontal ligament cells. This may have affected their capacity to migrate onto curetted root surfaces, thus allowing granulation tissue from bone to populate the surface and cause resorption and ankylosis. Similarly, in a beagle dog fenestration wound model, Aukhil *et al.* (1986) found no difference in healing between demineralized versus non-demineralized sites using citric acid.

The effects of citric acid, tetracycline or sterile saline, with or without nonresorbable membranes were further evaluated in a mid-root fenestration wound model in dogs (Sammons *et al.*, 1990). Radioautography revealed cell proliferation inhibition at one day post-wounding with little difference between the experimental groups and sterile saline controls. Light microscopy results suggested that during the initial stage of healing, the periodontal ligament is the principle source of fibroblastic proliferation and extracellular matrix production. In addition, the citric acid treated sites demonstrated the greatest amount of cementogenesis while the tetracycline treated sites demonstrated the greatest deposition of bone (Hamilton *et al.*, 1990). No differences in periodontal vascular response were seen in membrane versus no membrane sites from 7 to 21

days. The coronal segment of the adjacent periodontal ligament contributed more to initial vascularization of the defects than did the apical or mid-fenestration areas. No advantages regarding vascular response were recognized with any of the experimental treatments after 7 days (Doctor *et al.*, 1990).

Nalbandian and Hellden (1980, 1982) evaluated the regenerative potential of the periodontium adjacent to polytetrafluoroethylene, utilizing a fenestration wound model in 3 Beagle dogs. A semilunar incision was made to gain access to create a midroot, 4 x 4 mm fenestration in facial alveolar bone. Subsequently, a 2 mm diameter, 1.5 mm deep, cylinder of polytetrafluoroethylene was fitted into a cavity of the same dimensions prepared into the center of the root surface. Despite highly variable healing, a few sites at the 57 and 91 day observation periods revealed new cementum on the walls and floor of the preparation. Bone repair was evident, nearly covering the implant in some sites. Cementum and bone had formed under the implant in one site. The authors concluded that alveolar bone, connective tissue and cementum can develop in close proximity to polytetrafluoroethylene. They further speculated that such a coating may be considered in the future as a potential protective seal for roots of replanted or transplanted teeth in an attempt to prevent ankylosis.

The ability of biodegradable membranes to facilitate guided tissue regeneration was recently evaluated in a fenestration wound model in monkeys (Quinones *et al.*, 1990). Polyglactin woven mesh barriers were placed over fenestration sites and healing compared to no-membrane control sites at one month. Experimental sites demonstrated new cementum, bone and an immature, fibrillar, vascular periodontal ligament, while the control sites had a connective tissue adhesion without new cementum or bone. These results led the authors to suggest that the polyglactin mesh facilitated regeneration of the periodontium. No specifics regarding fenestration diameter or depth of periodontium removal were provided.

E. Summary of Fenestration Wound Healing Studies

The fenestration wound model has demonstrated the potential to heal by regeneration of the lost periodontium. Wounding of the periodontium results in stimulation of mitotic activity in adjacent cells (Gould *et al.*, 1980). Cells located within approximately 200 um of the surgically created fenestration undergo mitosis to repopulate the wound. In addition, paravascular progenitor cells were found to be located in the periodontal ligament within 5 um of blood vessels (Gould *et al.*, 1980). These cells apparently undergo migration 3 to 7 days after wounding and undergo mitosis either before or during migration (Iglhaut *et al.*, 1988).

Membranes have been utilized in the fenestration model in an attempt to form a barrier (Gould *et al.*, 1980) to eliminate any potential influence from the connective tissue of the mucoperiosteal flap and to encourage repopulation of the wound with cells from the adjacent periodontal ligament. Membranes create a "space" (Gould *et al.*, 1980, Nyman *et al.*, 1982) to facilitate repopulation of the experimental site. Membranes by themselves do not necessarily increase the mitotic activity of adjacent cells but may assist in directing and selecting various cell types for repopulation (Aukhil and Iglhaut 1988). When comparing results of various fenestration model studies, it appears that a membrane is not necessary to achieve regeneration of the experimentally removed periodontium. Examiners have found no difference in healing between sites with and without membranes (Doctor *et al.*, 1990). In addition, membranes do not appear to adversely affect healing in a fenestration type wound healing model.

There is a variability of results among studies. The latter is due to a lack of standardization of study design between studies. Fenestration size ranges from 0.8 mm to 5 to 7 mm, while several studies failed to report the size of the fenestration. Several animal models, including mice, rats, rabbits, dogs, monkeys and humans have been utilized. Variable results have been reported between various species with identical

study designs (Register 1973). Given the aforementioned variables, it is difficult to make correlations between studies.

The fenestration wound model has obvious advantages over other models. These include: 1) utilization of a normal non-diseased periodontium, 2) minimizing the effects of potential periodontopathic microorganisms, 3) decreasing anatomical variables, 4) minimizing hygiene variables, 5) minimizing the effects of other local factors and 6) retaining the tooth in function, thus preserving occlusal forces. Although problems do occur with the fenestration model, other models have problems that are eliminated with the fenestration model. For example, despite similarities of resected crown/submerged root models, these do not retain function of the tooth, thus eliminating occlusal forces and their potential influence on the healing periodontium. In addition, fenestration of the soft tissue over the submerged root may occur. Models that communicate with the oral cavity (i.e., experimentally produced and naturally occurring periodontitis models) are valuable in examining disease progression and wound healing. However, the use of root conditioning agents as well as other adjunctive treatment modalities and their influence on wound healing should be initially evaluated in the absence of the numerous variables present in such a model.

F. STATEMENT OF THE PROBLEM

The fenestration wound model has been utilized in several previous studies, but minimal documentation exists in the standardization of this model. The primary goal of this pilot project is to develop and define a fenestration wound model to study periodontal wound healing events. To accomplish this goal, this model will be utilized to evaluate effects on healing by various treatment modalities. Secondary goals consist of the evaluation of these treatment modalities. Healing adjacent to titanium implant materials, resorbable and non-resorbable epithelial exclusion membranes and resorbable membranes incorporated with growth factor are evaluated.

III. MATERIALS AND METHODS

A. Animals

Four, 7 year old adult male monkeys (*Macaca mulatta*) ranging in weight from 10 to 15 kilograms were obtained from the Experimental Animal Research Center, Brooks Air Force Base, Texas. All monkeys were quarantined for 12 weeks upon arrival before any experimental procedures were initiated. Monkeys were individually housed and fed a soft chow and water *ad libitum*.

B. Surgical Techniques

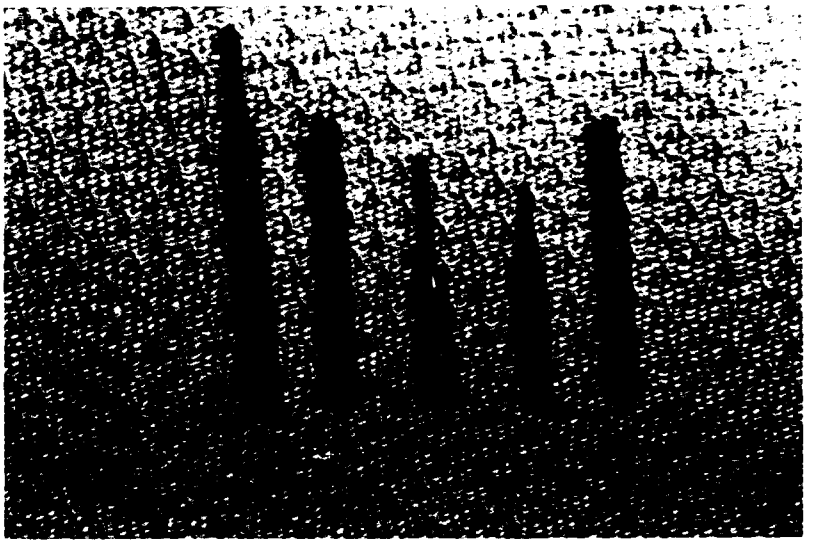
The monkeys were sedated with intramuscular injections of Ketamine (5-10 mg/kg body weight), Acepromazine (0.1-0.2 mg/kg body weight), and Atropine sulfate (0.01-0.05 mg/kg body weight). A thorough intraoral examination including periodontal probing was accomplished to assure no periodontal disease, other than gingivitis, existed. The teeth were scaled to remove supragingival calculus. The initial surgical procedure was performed one week after the examination and scaling phase.

Prior to the surgical procedure, the animals were sedated as previously described and surgical level anesthesia maintained with fluothane and oxygen. The mucous membrane in one arch/animal was infiltrated with 2% lidocaine containing 1:100,000 epinephrine. Facial sulcular incisions were made and facial mucoperiosteal flaps reflected in each sextant. A 2.5 mm diameter trephine at slow speed with a continuous sterile saline drip was used to make fenestration wounds at approximately the mid-root level of eight roots per arch (**Plates 1 A, B and 2 A, B**). Hand instruments were used to remove the boney plug and periodontal ligament in the wound site and to refine the margin of the fenestration. A quarter round bur was used as a pilot drill to initiate the root preparation in the center of the fenestration. Subsequently, a straight fissure bur was utilized to remove cementum and dentin to a depth of approximately one to two millimeters in the root surface at the non-titanium sites (**Plate 3 A and B**). Further preparation of the root surface accompanied placement of a titanium screw in four to

Plate 1

A. Rotary instruments utilized to create fenestrations and preparations for titanium screws. From left to right: trephine, pilot one-half round, straight fissure, countersink and tap.

B. Titanium screw



A

B

Plate 2

A. Use of a trephine to create a fenestration over the facial root surface three to five millimeters apical to the alveolar crest.

B. Removal of a boney plug and periodontal ligament from a fenestration site following use of a trephine.

Plate 3

A. A pilot drill used to locate the center of a fenestration and to initiate root preparation.

B. Straight fissure bur used for root preparation demonstrating removal of cementum and dentin. One millimeter preparations were created at non-titanium sites and two millimeter preparations at titanium sites.

six of the eight sites per arch. This consisted of extending the root preparation to the depth of the titanium screw length. The screws were pure titanium with a head diameter of 1.4 mm and a length of 2.0 mm. A tap was utilized to create a threaded surface within the site. Subsequent countersinking allowed the screw head to become flush with the root surface (**Plate 4 A and B**). All procedures utilized a continuous sterile saline drip to avoid overheating of the surrounding tissues. The screw was then placed in the prepared site until it was tight and even with the outer root surface (**Plate 5 A and B**). Subsequently, a 100 micron thick polytetrafluoroethylene membrane (PTFE) with a 0.2 micron pore diameter was placed over the fenestration in four of the eight sites per arch: two or three of the titanium screw sites and one or two of the non-screw control sites (**Plate 6 A**). The membrane was cut so that it would cover the wound and overlap the marginal alveolar bone by 2 to 3 mm. One or two control sites were created per arch. The flaps were repositioned and sutured with 4-0 plain gut suture. Intramuscular Combiotic antibiotics (200,000 units) were given daily for five days after surgery. Two weeks subsequent to surgery and weekly thereafter the animals were sedated, the teeth scaled and a 0.12% solution of chlorhexidine gluconate was topically applied to all teeth.

Fifteen weeks after the initial surgery, a second surgical procedure was performed in the opposite arch of each animal. Fenestration sites were produced as described for the initial surgery. Four of the eight sites per arch received titanium screws similar to the initial surgery. A collagen membrane with or without Transforming Growth Factor-ß1 (TFG-ß1) was placed over all fenestration sites so that it would cover the wound and overlap the marginal alveolar bone by 2 to 3 mm (**Plate 6 B**). The flaps were repositioned and sutured with 4-0 silk sutures. Intramuscular combiotic antibiotics (200,000 units) were given daily for seven days after surgery. The animals were sedated at one week and sutures were removed. The teeth were debrided and a 0.12% solution of chlorhexidine gluconate was topically applied to all teeth weekly for the remainder of the study period.

Plate 4

A. Tap instrument used to prepare sites to receive the titanium screws.

B. Countersink instrument facilitated seating of titanium screw so that the screw head was flush with the surrounding root surface.

Plate 5

A. Placement of titanium screw.

B. Titanium screw in place and flush with the surrounding root surface.

Plate 6

A. PTFE membrane placed to extend two to three millimeters beyond fenestration wound periphery.

B. Collagen membrane placed to extend two to three millimeters beyond two fenestration wound sites.

The collagen membranes were provided by Collagen Corporation. They were 60 um thick and consisted of 95% Type I, 5% Type III pepsin solubilized bovine dermal collagen. Ten percent of the collagen was chemically cross-linked. Bovine bone TGF-ß1 was incorporated in one-half of the collagen membranes during processing at a concentration of 2.5 ug TGF-ß1 per square centimeter of membrane.

C. Tissue Preparation

Five weeks after the second surgery or 20 weeks after the initial surgery the animals were sedated using Ketamine and Acepromazine. General anesthesia was administered with 120 mg Pentobarbital. Sacrifice was accomplished with 125 mg T-61 Euthanasia solution and the carotid arteries were subsequently perfused with 10% formalin. The mandibles and maxillae were dissected and immersed in 10% formalin. Tissue blocks consisting of individual teeth containing experimental sites were made and transported in 10% formalin to the University of Gothenburg, Sweden, for processing. The non-titanium sites were decalcified in EDTA for 14 days, vacuum embedded in paraffin and sectioned in a microtome giving 3 to 5 micrometer thick sections. The sections were then dehydrated in serial alcohol solutions and stained with either hematoxylin and eosin or with van Gieson stains. Sites that had received titanium screws were embedded in acrylic resin and subsequently underwent a grinding technique to obtain one 10 micrometer section per site nearest the middle of the screw. Titanium screw sections were stained with either toluidine blue or Masson's-Trichrome-Goldner stain. All stained sections were examined by light microscopy for the presence or absence of bone, periodontal ligament and/or cementum in each site.

IV. RESULTS

A. Twenty Week Wound Variables

Wounds were created in a total of 64 sites in the four monkeys. The thirty-two wound sites evaluated for 20 weeks included: 22 sites with titanium screws, 11 of these sites received PTFE membranes; 10 additional sites served as non-titanium controls, 5 of which received PTFE membranes. ((**Table 1**) Wound sites listed by appropriate tooth: international numbering system, name of tooth and applicable root(s).)

TABLE 1

Twenty Week Fenestration Wound Site Treatment Modalities

ANIMAL #368 - MANDIBULAR ARCH

35 LEFT 2ND PREMOLAR	- SCREW, PTFE MEMBRANE
34 LEFT 1ST PREMOLAR MESIAL ROOT	- SCREW ONLY
32 LEFT LATERAL INCISOR	- PTFE MEMBRANE ONLY
31 LEFT CENTRAL INCISOR	- SCREW ONLY
41 RIGHT CENTRAL INCISOR	- SCREW, PTFE MEMBRANE
42 RIGHT LATERAL INCISOR	- CONTROL (HOLE ONLY)
44 RIGHT 1ST PREMOLAR MESIAL ROOT	- SCREW, PTFE MEMBRANE
45 RIGHT 2ND PREMOLAR	- SCREW ONLY

ANIMAL #376 - MAXILLARY ARCH

17 RIGHT 2ND MOLAR DISTOFACIAL ROOT	- SCREW & PTFE MEMBRANE
17 RIGHT 2ND MOLAR MESIOFACIAL ROOT	- SCREW ONLY
12 RIGHT LATERAL INCISOR	- PTFE MEMBRANE ONLY
11 RIGHT CENTRAL INCISOR	- SCREW ONLY
21 LEFT CENTRAL INCISOR	- SCREW & PTFE MEMBRANE
22 LEFT LATERAL INCISOR	- CONTROL (HOLE ONLY)
27 LEFT 2ND MOLAR MESIOFACIAL ROOT	- SCREW & PTFE MEMBRANE
27 LEFT 2ND MOLAR DISTOFACIAL ROOT	- SCREW ONLY

ANIMAL #386 - MAXILLARY ARCH

17 RIGHT 2ND MOLAR DISTAL ROOT	- CONTROL (HOLE ONLY)
17 RIGHT 2ND MOLAR MESIAL ROOT	- PTFE MEMBRANE ONLY
12 RIGHT LATERAL INCISOR	- SCREW ONLY
11 RIGHT CENTRAL INCISOR	- SCREW, PTFE MEMBRANE
21 LEFT CENTRAL INCISOR	- SCREW ONLY
22 LEFT LATERAL INCISOR	- SCREW, PTFE MEMBRANE
27 LEFT 2ND MOLAR MESIAL ROOT	- CONTROL (HOLE ONLY)
27 LEFT 2ND MOLAR DISTAL ROOT	- PTFE MEMBRANE ONLY

ANIMAL #406 - MAXILLARY ARCH

15 RIGHT 2ND PREMOLAR	- PTFE MEMBRANE ONLY
14 RIGHT 1ST PREMOLAR	- SCREW ONLY
12 RIGHT LATERAL INCISOR	- SCREW & PTFE MEMBRANE
11 RIGHT CENTRAL INCISOR	- SCREW ONLY
21 LEFT CENTRAL INCISOR	- SCREW & PTFE MEMBRANE
22 LEFT LATERAL INCISOR	- SCREW ONLY
27 LEFT 2ND MOLAR MESIOFACIAL ROOT	- SCREW & PTFE MEMBRANE
27 LEFT 2ND MOLAR DISTOFACIAL ROOT	- CONTROL (HOLE ONLY)

B. FIVE WEEK RESULTS

Thirty-two sites were evaluated at 5 weeks and included: 16 sites with titanium screws, 8 of these sites received collagen membranes with TGF-ß1, while the other 8 sites received collagen membranes with no growth factor; 16 additional sites served as non-titanium controls, 8 of which received collagen membranes with TGF-ß1, while the other 8 sites received collagen membranes without TGF-ß1. (**Table 2**)

TABLE 2

Five Week Fenestration Wound Site Treatment Modalities

ANIMAL #368 - MAXILLARY ARCH

15 RIGHT 2ND PREMOLAR	- COLLAGEN MEMBRANE
14 RIGHT 1ST PREMOLAR	- SCREW, COLLAGEN MEMBRANE
12 RIGHT LATERAL INCISOR	- COLLAGEN MEMBRANE + TGF-ß1
11 RIGHT CENTRAL INCISOR	- SCREW, COLLAGEN MEMBRANE + TGF-ß1
21 LEFT CENTRAL INCISOR	- COLLAGEN MEMBRANE + TGF-ß1
22 LEFT LATERAL INCISOR	- SCREW, COLLAGEN MEMBRANE + TGF-ß1
24 LEFT 1ST PREMOLAR	- COLLAGEN MEMBRANE
25 LEFT 2ND PREMOLAR	- SCREW, COLLAGEN MEMBRANE

#376 - MANDIBULAR ARCH

37 LEFT 2ND MOLAR MESIAL ROOT	- COLLAGEN MEMBRANE
35 LEFT 2ND PREMOLAR	- SCREW, COLLAGEN MEMBRANE
32 LEFT LATERAL INCISOR	- COLLAGEN MEMBRANE + TGF-ß1
31 LEFT CENTRAL INCISOR	- SCREW, COLLAGEN MEMBRANE + TGF-ß1
41 RIGHT CENTRAL INCISOR	- COLLAGEN MEMBRANE + TGF-ß1
42 RIGHT LATERAL INCISOR	- SCREW, COLLAGEN MEMBRANE + TGF-ß1
45 RIGHT 2ND PREMOLAR	- COLLAGEN MEMBRANE
47 RIGHT 2ND MOLAR MESIAL ROOT	- SCREW, COLLAGEN MEMBRANE

#386 - MANDIBULAR ARCH

37 LEFT 2ND MOLAR MESIAL ROOT	- SCREW, COLLAGEN MEMBRANE + TGF-ß1
35 LEFT 2ND PREMOLAR	- COLLAGEN MEMBRANE + TGF-ß1
32 LEFT LATERAL INCISOR	- SCREW, MEMBRANE
31 LEFT CENTRAL INCISOR	- COLLAGEN MEMBRANE
41 RIGHT CENTRAL INCISOR	- SCREW, COLLAGEN MEMBRANE
42 RIGHT LATERAL INCISOR	- COLLAGEN MEMBRANE
45 RIGHT 2ND PREMOLAR	- COLLAGEN MEMBRANE + TGF-ß1
47 RIGHT 2ND MOLAR MESIAL ROOT	- SCREW, COLLAGEN MEMBRANE + TGF-ß1

#406 - MANDIBULAR ARCH

37 LEFT 2ND MOLAR MESIAL ROOT	- SCREW, COLLAGEN MEMBRANE + TGF-ß1
35 LEFT 2ND PREMOLAR MESIAL ROOT	- COLLAGEN MEMBRANE + TGF-ß1
32 LEFT LATERAL INCISOR	- COLLAGEN MEMBRANE
31 LEFT CENTRAL INCISOR	- COLLAGEN MEMBRANE
41 RIGHT CENTRAL INCISOR	- SCREW, COLLAGEN MEMBRANE
42 RIGHT LATERAL INCISOR	- SCREW, COLLAGEN MEMBRANE
45 RIGHT 2ND PREMOLAR MESIAL ROOT	- COLLAGEN MEMBRANE + TGF-ß1
47 RIGHT 2ND MOLAR MESIAL ROOT	- SCREW, COLLAGEN MEMBRANE + TGF-ß1

C. Clinical Results

Following the first surgical wounding procedure, three animals (#376, #386, #406) demonstrated some apical migration of the flaps. This was most evident in the posterior sextants. Four PTFE membranes were clinically exposed 2 weeks after wounding (**Plate 7 A and B**). These areas developed clinical infections with purulence and localized erythema. The exposed membranes were trimmed weekly to assure that they remained level or slightly below the gingival surface until the sites either healed or the membrane was exfoliated. Three of the sites demonstrated progressive membrane exposure until the membranes were eventually trimmed to the point that they were removed or no longer clinically visible (#406 site 27 - 6 weeks; #406 site 15 and #386 site 17 Mesial - 8 weeks). One of these sites, #406 site 15, developed a fistula which remained at sacrifice (**Plate 8 A and B**). Histologically, this site demonstrated retention of a portion of the PTFE membrane and a giant cell reaction adjacent to its surface. The membrane in site #386 site 17 exfoliated between 2 and 3 weeks as the site appeared clinically normal at this time period and no membrane was detected during histologic examination. The remaining 20 week sites healed uneventfully (**Plate 9 and B**).

Subsequent to the second wounding procedure, the flaps in two sites demonstrated an apical migration, but no exposure of membranes was clinically apparent (**Plate 10 A and B**). All other sites demonstrated minimal or no apical migration of the flaps. All collagen membrane sites healed uneventfully without any clinical signs of infection (**Plate 11 A and B**).

Of the non-titanium sites, six were lost due to processing errors. These included three of the 20 week sites, two control - "hole only" sites (#368 site 42, #386 site 17D), and one PTFE membrane site (#386, site 27D). The additional three sites were 4 week specimens. All of the latter had been treated with collagen membranes without growth factor (#376 site 37M, #386 site 31, #406 site 32).

Plate 7

A. Two week post surgical PTFE membrane site demonstrating subsequent apical positioning of flap. Exposure of the PTFE membrane can be seen facial to the premolars extending 1 to 2 mm coronal to the surgical flap margin.

B. Progressive exposure of PTFE membrane was noted four weeks post surgery.

Plate 8

A. Same site as in Plate 7 with membrane trimmed level with tissue margin (four weeks post surgery).

B. Same site 20 weeks post surgery. An abscess is present facial to the second premolar. Note the uneventful healing of adjacent areas.

Plate 9

A. PTFE membrane sites two weeks post surgery. Minimal recession of flap has occurred.

B. Same site twenty weeks post surgery which had demonstrated no post-operative sequelae.

Plate 10

A. Collagen membrane site one week post surgery demonstrating apical movement of the surgical flap.

B. Same site two weeks later.

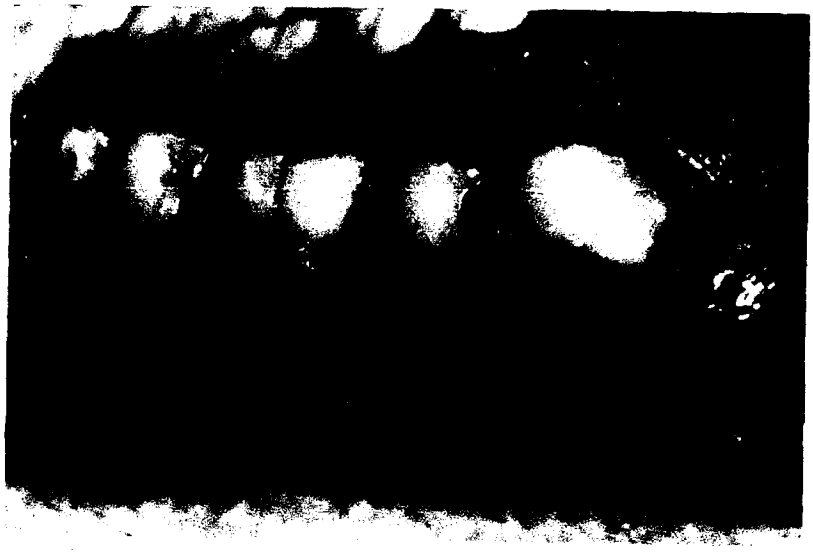
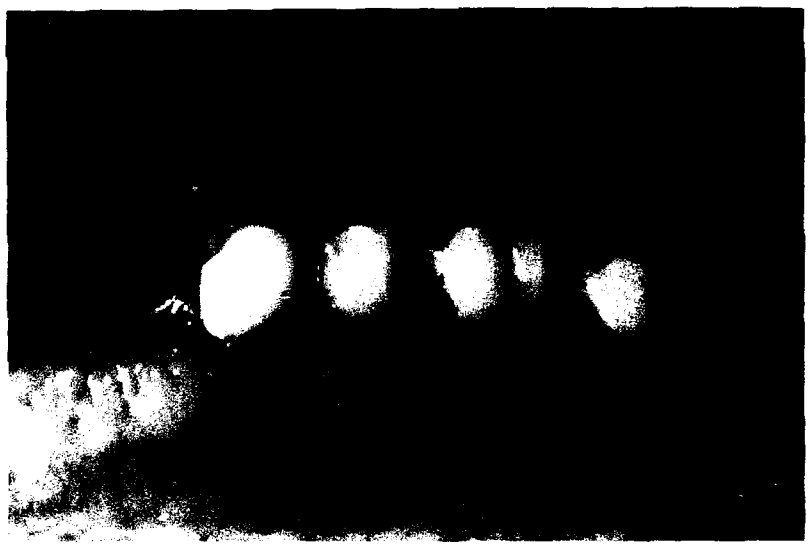


Plate 11

A. Collagen membrane sites one week post surgery. Note minimal flap recession.

B. Same site three weeks post surgery.



D. Histological Results

1. Twenty week control "hole only" sites

The twenty week control "hole only" wounds demonstrated deposition of a uniform layer of new cementum in close apposition to the surgically exposed dentin (**Plates 12 A, B and 13 A, B**). In some sections, the cementum layer was not completely removed from within the trephine outline. A new cemental layer formed over both the old cementum and denuded dentin in these sections (**Plate 13 A and B**). A perpendicular oriented periodontal ligament of varying thickness inserted into the new cemental layer and overlying bone which had completely regenerated over the wound site. The overlying bone often extended within the defect area, but was always separated from the cementum by a functionally oriented periodontal ligament. The thickness of the periodontal ligament and the extent to which the new bone extended within the defect varied with the depths of the individual defects. The deeper the defect the greater the extension of bone within the area.

2. Twenty week PTFE membrane non-titanium sites

The twenty week PTFE membrane covered wounds demonstrated a similar appearance as the control sites with complete regeneration within the experimental sites (**Plate 14 A**). Five sites received PTFE membranes over the experimental wounds. One of these sites (#386 site 27D) was lost during processing. Two additional sites had the membrane clinically exposed by one week post-surgically, one of which was partially removed (#406 site 15) during the healing period and one (#386 site 17M) which exfoliated 2 to 3 weeks post-surgery. All wound sites appeared similar whether or not the membrane was retained throughout the study. A uniform layer of new cementum was present in close apposition to surgically exposed dentin. A perpendicular oriented periodontal ligament of varying thickness separated the new cemental layer and the overlying bone. New bone had completely regenerated over the wound site and often extended into the defect when the defect was large. Variability of defects were

Plate 12

A. A twenty week control "hole only" site. New cementum formation (C) can be seen over previously denuded dentin (D), with perpendicular fiber formation (P) and boney fill (B) of the fenestration site. (van Gieson, magnification x 40)

B. Same site. (van Gieson, magnification x 100)

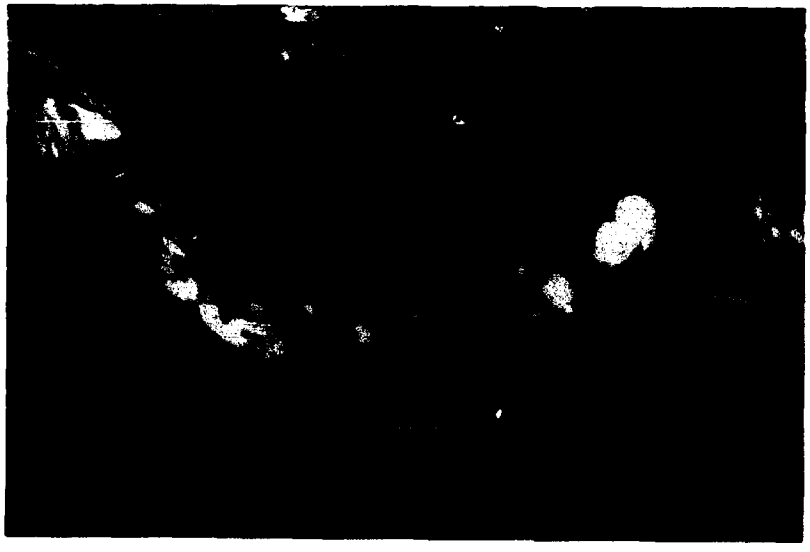
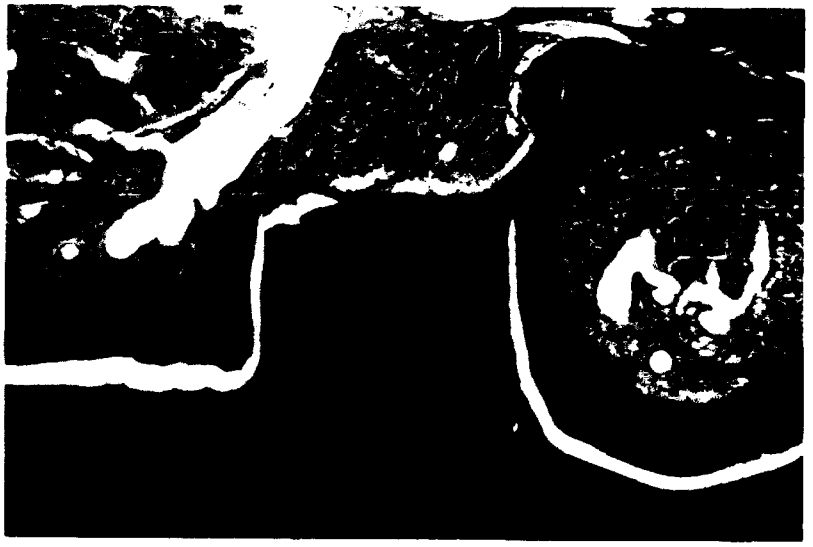


Plate 13

A. A twenty week control "hole only" site. New cementum formation (C) can be seen over previously denuded dentin (D) and old cementum with perpendicular fiber formation (P) and boney fill (B) of the fenestration site. (van Gieson, magnification x 40)

B. Same site. (van Gieson, magnification x 100)



primarily due to differences in depth. Some wounds extended into the pulp chamber and occasionally to the opposing surface of the tooth to communicate with the opposing periodontium. Regeneration was present on the opposing surface of these sites. Pulpal exposures were often bridged with reparative dentin. New cementum formation extended over these dentinal bridges. A functionally oriented periodontal ligament was interposed between the new bone and cementum. The pulps contained histologically vital cells.

The PTFE membrane was separated from the alveolar bone surface by a dense layer of fibrous connective tissue in the three sites retaining some or all of the membrane. In addition, all three sites contained numerous multinucleated giant cells adjacent to the PTFE membranes (**Plate 14 A and B**). One of these three sections had the membrane clinically exposed by two weeks post surgery and demonstrated a purulent exudate during the course of the study. Although these sites demonstrated a giant cell response to the PTFE membrane, these fenestration wounds demonstrated complete regeneration similar to the control sites.

3. Twenty week titanium sites with and without PTFE membranes

The twenty week titanium sites exhibited wide variability in healing, probably related to technical difficulties encountered in the attempt to precisely place the titanium screws. No differences could be ascertained between sites with or without PTFE membranes. The PTFE membrane in one site (#406 site 27M) was exposed within two weeks after placement. Due to the processing procedure, only one histological section was available per site. Several sites demonstrated apparent cementum formation adjacent to the titanium surface (**Plates 15 A, B and 16 A, B**). Cementum often formed between the prepared dentin surface and the screw threads, occluding any space between the screw and the dentin surface. Cementum formed adjacent to the

Plate 14

A. A twenty week PTFE membrane site. New cementum (C), mature periodontal ligament formation (P) and boney fill of the fenestration are present. PTFE membrane (T) is intact and separated from the alveolar surface by fibrous connective tissue. (H & E, magnification x 40)

B. Magnification of PTFE membrane (T) of the same site. Note the confluence of adjacent multinucleated giant cells. (H & E, magnification x 250)

Plate 15

A. A twenty week titanium screw site with no membrane. New cementum (C) formation covers the screw head and has filled the area adjacent to the screw thread. A periodontal ligament is present (P) and bone (B) occludes the fenestration. (Magnification x 10)

B. Magnification of the above screw head. Note the close approximation of cementum (C) with the titanium (T) surface. (Magnification x 40)


P


P

Plate 16

A. A twenty week titanium screw plus PTFE membrane site. New cementum (C) has formed adjacent to the screw and partially over the screw head. New bone (B) occludes the fenestration and is separated from the new cementum and titanium surface with a functionally oriented periodontal ligament (P). The PTFE membrane is present and is separated from the bone surface by a layer of fibrous connective tissue. Note that the screw extends through the root to the opposite side. (Magnification x 10)

B. Magnification of screw head. (Magnification x 20)


B
P

screw head, often filling the groove of the screw head or extending to partially or completely cover the screw head surface in the representative section. A functionally oriented periodontal ligament was present between the cementum surface and newly formed alveolar bone. New bone bridged the wound surface. Relatively large formations of cementum were often present between the screw head and root surface, these areas apparently had a discrepancy between the titanium/root surface at placement. Screws often extended through the root surface, often resulting in internal root fracture. The precision of placement was variable and the perimeter of the screw head was often not confluent with the adjacent root surface (**Plate 17**). A layer of fibrous connective tissue was consistently present separating the PTFE membrane and the alveolar surface.

4. Five week collagen membrane non-titanium sites

Eight non-titanium wound sites received collagen membranes which overlapped the alveolar bone adjacent to the fenestration. Three sites were lost during processing. The remaining five sites demonstrated a newly formed cemental layer over the prepared dentinal surface and a periodontal ligament was present adjacent to the cementum. The periodontal ligament had a variable orientation with Sharpy's fibers inserting within the cemental surface. New alveolar bone occluded the fenestration in all specimens (**Plate 18 A**). The collagen membrane was often in direct contact with the alveolar surface resulting in the direct blending of the membrane and bone interface (**Plate 18 B**). With the exception of one site (#376 site 45) no inflammation was present adjacent to the sites or membranes. Site #376 site 45 had a pulpal exposure and the fenestration extended through the root to the adjacent surface. Diffuse inflammation was present on the buccal surface, but cementum formed over most of the exposed dentin. In addition, bone filled the fenestration site and periodontal ligament was present between the cementum and bone. No membrane was visualized in the site.

Plate 17

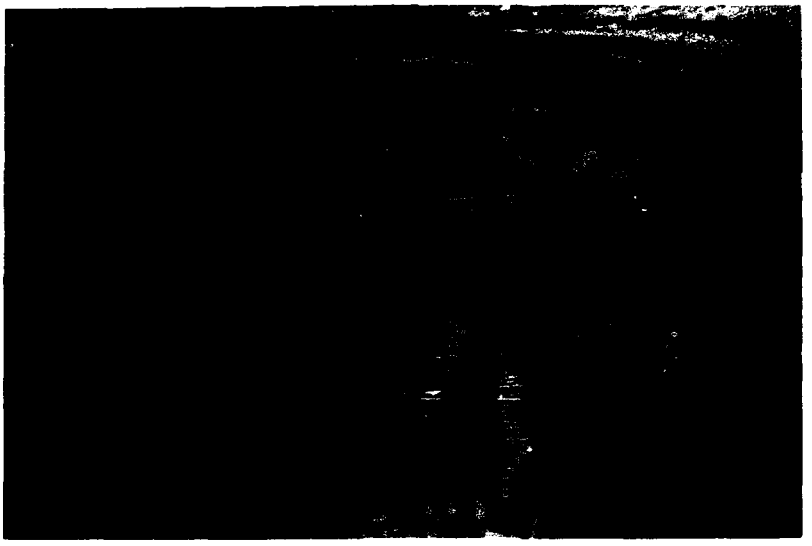
A. A twenty week titanium screw plus PTFE membrane site. New cementum formation aligns the screw threads and partially over the screw head. Note the malalignment of the screw causing a portion of the screw to extend beyond the root surface. (Magnification x 6)

B. Magnification of screw / root surface. (Magnification x 40)

Plate 18

A. A five week collagen membrane (without growth factor) site. New cementum (C) lines the fenestration and mixed orientation fiber (P) formation extends from the cementum to newly formed alveolar bone (B). The collagen membrane (M) is intact and is in close approximation to the bone surface. (van Gieson, magnification x 40)

B. Collagen/bone interface. (van Gieson, magnification x 100)



5. Five week collagen membrane with TFG-ß1 non-titanium sites

Eight non-titanium wound sites received collagen membranes containing 2.5 ug TGF-ß1 per square centimeter. All sites were available for histological examination. All sites consistently demonstrated a uniform layer of new cellular cementum adjacent to the surgically exposed dentin. A variable thickness periodontal ligament with a mixed orientation was present. Periodontal ligament fibers inserted into cementum and new alveolar bone overlayed and occluded the fenestration site (**Plate 19 A**). Collagen membrane was present and in direct contact with the alveolar surface at most sites, with surface melding comparable to the collagen membrane without growth factor sites (**Plate 19 B**). Although most sites demonstrated an apparent biocompatible association between the membrane and adjacent tissues, at least one site (#368 site 15) demonstrated multinucleated giant cells adjacent to the membrane (**Plate 20**). No apparent clinical exposure of the membrane or alteration in clinical healing was seen. An attempt was made in one site (#368 site 12) to fill the fenestration with the membrane. It was impossible to fully occlude the fenestration due to the tendency of the membrane to retain its original shape. However, portions of the membrane were retained within the fenestration and an additional membrane was placed over the site. The effected site demonstrated cementum formation over a portion of the exposed dentin within the fenestration site (**Plate 21 A**). The membrane appeared to be in direct contact and "blended" with the cemental surface in isolated areas (**Plate 21 B**). No bone extended within the site, and there was no apparent alveolar bone formation covering the fenestration site. The membrane appeared to be forming a barrier between the apical and coronal margins of the alveolar bone. The apical and coronal marginal interface of the boney fenestration and the interposing membrane had manifested direct contact or blending of the membrane and alveolar bone surfaces.

Plate 19

A. A five week collagen membrane plus TGF-ß1 site. New cementum (C) lines the fenestration and mixed orientation fiber (P) formation extends from the cementum to newly formed alveolar bone (B). The collagen membrane (M) is intact and is in close approximation to the bone surface. (van Gieson, magnification x 40)

B. Magnification of collagen/bone interface. (van Gieson, magnification x 100)

Plate 20

A five week collagen membrane plus TFG-β1 site. The membrane is partially separated from the bone surface and multinucleated giant cells can be seen adjacent to the collagen membrane.

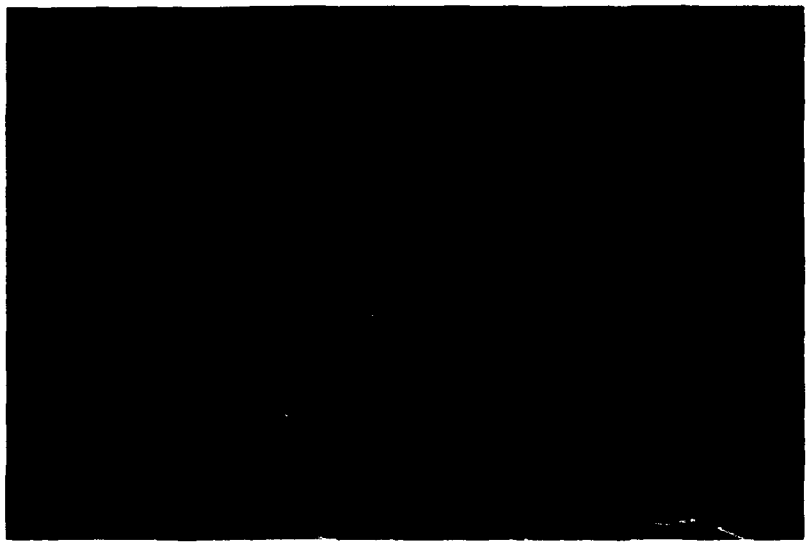
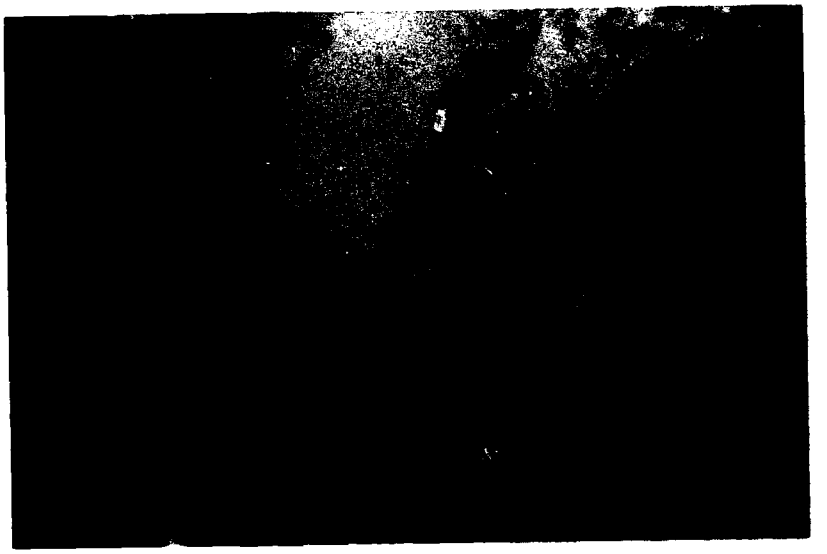


Plate 21

A. A five week collagen membrane plus TGF-ß1 site. The membrane was forced into the fenestration in an attempt to occlude the preparation. New cementum partially lines the denuded dentin and a mixed fiber orientation is present. No bone formation is present in the fenestration. (van Giesen, magnification x 40)

B. Collagen membrane (M) / cementum (C) interface. (van Giesen, magnification x 250)



6. Five week collagen membrane titanium sites

Eight sites received titanium screws within root surface preparations and a collagen membrane without g' wth factor over the fenestration sites. Isolated areas of cementum formation were present adjacent to the surgically exposed dentin but no cementum formation was observed adjacent to the titanium surface. A diffuse inflammatory infiltrate was often present adjacent to these sites.

7. Five week collagen membrane with TGF-ß1 titanium sites

Eight sites received titanium screws within root surface preparations and a collagen membrane containing 2.5 ug TGF-ß1 per square centimeter over the fenestration sites. A histologic picture similar to the titanium sites with membranes without growth factor was seen (**Plate 22 A and B**).

E. STATISTICAL ANALYSIS

Statistical analysis of healing parameters was not necessary as no histologic differences were seen between treatment modalities. All sites demonstrated similar stages of healing. No differences were observed between twenty week non-titanium sites with or without PTFE membranes. No differences were seen between twenty week titanium sites with or without PTFE membranes. No differences were noted between five week non-titanium sites plus collagen membrane with or without TGF-ß1. Finally, no differences were seen between five week titanium sites plus collagen membrane with or without TFG-ß1.

Plate 22

A. A five week collagen membrane plus TGF-ß1 plus titanium screw site. New cementum has formed on the denuded dentin surface and no cementum is seen adjacent to the titanium screw. Inflammation is present within the fenestration. (Magnification x 10)

B. Magnification of root / titanium interface. (Magnification x 40)

V. DISCUSSION

The fenestration wound model in this pilot study has proven to be an acceptable means of evaluating healing response to materials proposed to have therapeutic potential for managing periodontal disease. Previously, the fenestration wound model has been utilized in animal models varying from rodents to primates. The anatomy of the teeth and periodontium in non-human primates is very similar to that found in humans (Page and Schroeder 1982). In addition, similarities in immunologic response and disease susceptibility make the non-human primate the most predictable experimental model (Navia 1977). The titanium screws used in this study dictated the size of the experimental animal model in this pilot project to have root surfaces of adequate dimensions to allow placement of the screws. This, in addition to the timely availability of the experimental animals led to the use of four non-human primates, *Macaca mulatta*. Requirements of future projects, dependent on parameters and materials examined, may be adequately served by other species.

Previous studies have indicated that regeneration is possible with this wound model (Beckwith *et al.,* 1927, Beckwith and Williams 1928, Beube 1947, Jansen *et al.,* 1955, Nalbandian and Frank 1980). The advantage of excluding a potentially hostile oral environment allows for assessment of various materials, such as growth factors, on the influence of either increasing the mitotic rate of resident cell populations and/or the chemotaxis of these cells to selectively repopulate the wounded periodontium. This fenestration wound model evaluates a non-diseased periodontium and has no apparent role in the evaluation of periodontal disease itself, but rather is valuable in the assessment of regenerative capabilities. Other models have similarly excluded the oral environment by submerging the roots beneath the gingiva. This has been achieved either by removing the crowns of teeth and covering them with soft tissue flaps or by extracting teeth, removing the crowns and replanting them either in the socket or within a space made within the alveolus. The fenestration wound model used in this study has

several advantages over these other submerged models: (1) The teeth remain in function; this may influence wound healing. (2) The orientation and maturation of new periodontal ligament fibers may be dependent on the forces acting upon the teeth (Basset 1962). (3) The experimental animal retains function and is thus less likely to develop problems with otherwise necessary changes in diet. (4) Primary closure is easily obtained, although apical positioning of tissues may occur post-surgical. (5) Root canal therapy is not necessary in this model, whereas such therapy has often been performed in submerged models.

Difficulties incurred in using this model were primarily related to the technical nature of one of the study parameters utilized in this pilot project, in particular the placement of the titanium screws. Although the size of screws utilized was extremely small and thus more tedious to use, their size dictated the use of an animal with a relatively large dentition. Roots were often dilacerated and narrow from a mesial-distal dimension. Pre-operative radiographs improved the ability to locate the root surface with the alveolar fenestration, however it was difficult to place the fenestration precisely over the facial surface of the root to allow ideal placement of the titanium screw. Screws were 2 mm long with a diameter of 1.4 mm. Ideally the root was prepared to receive the screw such that the head of the screw would be flush or slightly countersunk with the root surface for its full diameter. Due to the limited root surface area available within the alveolar fenestration, the screw was often positioned so a portion of the screw head would be flush or countersunk and the remainder would be above the root surface. This was most notable at the mesial or distal line angles. In addition, many sites had obvious pulpal penetration. This was demonstrated clinically during preparation as loss of resistance to the bur could be felt, thus penetrating the pulpal space. From a histological examination, several wound sites were observed to communicate with the pulp chamber. This had no obvious clinical detriment as the animals demonstrated no signs or symptoms of distress. Histologically, most sites healed uneventfully,

demonstrating a dentin bridge over the exposure and cementum over the dentin. The titanium screw sites also demonstrated occasional fracture of the teeth which apparently was associated with screw placement. No clinical problems were encountered in these sites. Although, the titanium sites demonstrated occasional placement technique difficulties, all other procedures were less technique sensitive and can be easily followed to evaluate future materials. Standardization of the procedure with graduated instruments (e.g., burs and trephines) would improve the congruity between wound sites and improve the relative evaluation effectiveness.

One of the secondary goals of this pilot project was to determine if cementum would form on a titanium surface. The healing times evaluated in this study were chosen based on this treatment modality. A twenty week healing time was arbitrarily chosen as the longest evaluation time for this parameter. The minimum evaluation period of five weeks was chosen based on previous fenestration studies demonstrating cementum formation in various species by 3 to 5 weeks (Beube 1947, Nalbandian and Frank 1980, Hellden 1972).

An interesting observation in this model was that all sites, except titanium sites, demonstrated similar levels of healing. The size of the defects in this study were apparently small enough that regeneration occurred no matter what treatment was utilized. This may be inherently related to the fenestration wound model. Quinones *et al.* (1990) reported finding no new bone or cementum formation in control sites utilizing a fenestration wound model in monkeys, while regeneration of the periodontium was seen with the use of a biodegradable polyglactin membrane over the sites. No mention was made of the fenestration diameter or depth of periodontium removed. Determination as to whether a critical size defect exists in the periodontal fenestration wound model has not been determined. A critical size defect is defined as a "defect of a size that will not heal during the lifetime of the animal" (Schmitz and Hollinger 1986). If

a critical size defect exists in this model, the effects of various materials on the size of that defect would be a quantitative means of evaluating these materials.

The relatively rapid reformation of crevicular epithelium subsequent to periodontal surgery decreases the potential of new attachment formation (Pritchard 1957). Bjorn (1961) concluded that, "By excluding epithelium from the healing process in the periodontium, it is possible to obtain complete mesenchymal reattachment with reorganization of root cementum, periodontal membrane and alveolar bone." Artificial membranes have been utilized to exclude the epithelium from the root surface and to allow the cells derived from the periodontal ligament adequate time to repopulate the root surface. New attachment formation has been documented histologically in humans utilizing membranes (Nyman *et al.*, 1982, Gottlow *et al.*, 1986, Stahl *et al.*, 1990). In addition to excluding epithelium, membranes have been utilized to create a "space" for the migration of periodontal ligament cells (Gottlow *et al.*, 1984). The migration of cells to fenestration wounds apparently occurs during the first week and is nearly completed by day 21. The use of membranes in the treatment of a diseased periodontium may effectively exclude the oral epithelium from populating the root surface prior to the formation of a new attachment (Nyman *et al.*, 1980, 1982; Karring *et al.*, 1980, 1984, 1985; Gottlow *et al.*, 1984 b). Although no histological differences were demonstrated between PTFE membrane and non-membrane sites in this study, the addition of membranes to the fenestration wound model may eliminate an additional variable in the clinical setting when their use may be planned with additional adjunctive treatment modalities. Experimental sites with and without membranes may prove valuable to reveal any synergistic effect a membrane may have with additional treatment modalities. The fenestration wound model used in this study naturally excludes epithelial ingrowth within the experimental sites without the use of membranes. However, PTFE membranes were utilized at some twenty week observation sites to exclude ingrowth of gingival connective tissue, to create a space and to allow selective repopulation of the

sites from the adjacent periodontal ligament and or bone cells. Granulation tissue from bone and gingival connective tissue has been proposed to induce root resorption (Melcher 1970, Line *et al.,* 1974, Karring *et al.,* 1980, Nyman *et al.,* 1980, Andreasen 1981, Boyko *et al.,* 1981, Lopez and Belvederessi 1983, Gottlow *et al* 1984, Karring *et al.,* 1984, Magnusson *et al.,* 1984, 1985). Although 50% of the twenty week sites in this project did not receive membranes to exclude the gingival connective tissue, minimal root resorption was demonstrated. This is in agreement with the findings of Nyman et al. (1982). In addition, no histological differences were seen between PTFE membrane and non-membrane sites. These findings may have been related to the defect size and/or the propensity of the adjacent cells to repopulate the wound. The defect size may have been small enough that the gingival connective tissue was isolated from the root surface by bridging of the flap over the defect. Alternatively, the size may have allowed for selective repopulation by adjacent cells in advance of cells from the gingival connective tissue. In future studies, critical defect size should be determined in this model to clarify these variables.

The PTFE membrane non-titanium sites demonstrated a foreign body giant cell reaction. Only photomicrographs were available for evaluation of PTFE membrane titanium sites, therefore it was difficult to evaluate the foreign body giant cell reaction in these sites. The sample size of sites demonstrating foreign body reaction was small and the membranes in two of the three sites demonstrating this reaction were clinically exposed shortly after their placement. However, one site remained covered and healed uneventfully, but demonstrated a similar foreign body giant cell reaction. Similar reactions have been reported with the use of Teflon Proplast (Vitek Inc.) in temporomandibular joint implants (Timmis *et al.,* 1986, Bronstein 1987, Kaplan *et al.,* 1988, Valentine *et al.,* 1989), hip joint implants (Charnley 1962), infraorbital Teflon (Proplast) implants (El Deeb and Holmes 1989), and Teflon (Proplast) implants placed in combination 2 and 3 wall periodontal osseous defects (Radell and Cassingham

1980). This reaction was hypothesized to be due to mobility within the area causing wear of the implant and subsequently creating loose particles of the implant material (Timmis *et al.*, 1986). Although giant cell reactions have previously been reported with the use of Teflon Proplast in periodontal defects, no prior reports have associated a giant cell reaction with the use of PTFE membranes. Despite this reaction in both sites with and without post-surgical exposure of membranes, wounds of the periodontium proceeded to heal without any apparent adverse effect. It is interesting to note that PTFE membranes consistently demonstrated fibrous connective tissue interposed between the membrane and the bone surface. This finding along with the foreign body giant cell reaction raises suspicion that these membranes may not be as biocompatible as previously proposed.

Although PTFE membranes have demonstrated successful exclusion of the gingival connective tissue and epithelium during periodontal healing, a second surgical procedure is necessary to remove them. Post-operative complications, including exposure of the membrane, abscess formation and increased tissue fragility during membrane removal add to the potential complications associated with their use in regenerative procedures. Biodegradable membranes have been successfully utilized to demonstrate regeneration of periodontal tissues. Membranes made of collagen (Pitaru *et al.*, 1987, 1988 a & b, 1989; Tanner *et al.*, 1988; Blumenthal 1988; West and Goldberg 1988; Kodama *et al.*, 1989; Minabe *et al.*, 1989; Pfeifer *et al.*, 1989; Chung *et al.*, 1990), polylactic acid (Magnusson *et al.*, 1988, 1990), ox cecum derivatives (Cargile membrane, Ethicon Co.) (Card *et al.*, 1989), freeze-dried homologous dura mater (Zaner *et al.*, 1989, Garrett *et al.*, 1990), and polyglactin (Fleisher *et al.*, 1988; Quinones *et al.*, 1990 a & b; Caton *et al.*, 1990; Kon *et al.*, 1991) have been evaluated.

Collagen is the major extracellular macromolecule of the periodontium. It is continually undergoing production and breakdown and thus is biodegradable. It has chemotactic properties for fibroblasts (Postelwaite *et al.*, 1978, Yaffe *et al.*, 1984) which

may influence the migration of periodontal ligament cells. Collagen has been used in humans and animals for several years and has proved to be a weak immunogen (Sableman 1985). In addition, varying success, utilizing collagen membranes, has been reported in preventing apical migration of oral epithelium (Pitaru *et al.*, 1987, 1989; Blumenthal 1988; Pfeifer *et al.*, 1989). If the coronal aspect of the membrane undergoes rapid resorption, a wedge of epithelium can potentially migrate between the lamina propria of the gingiva and the coronal segment of the membrane (Pitaru *et al.*, 1989). Resorption properties can be altered by varying the amount of cross-linkage in the membrane, allowing longer periods of epithelial exclusion and improved success for regeneration (Pfeifer *et al.*, 1989, Minabe *et al.*, 1989). Pfeifer *et al.* (1989) utilized cross-linked and non-cross-linked bovine collagen membranes in Grade II furcas in Beagle dogs. The cross-linked collagen membranes degraded between 6 and 8 weeks and successfully prevented the downgrowth of epithelium, while the non-cross-linked membranes where resorbed between 1 and 3 weeks and exhibited healing similar to control sites. A fibrous connective tissue band separated the cross-linked membrane and the previously denuded bone at 1, 3, 4, and 8 week sections. Similarly, Pitaru *et al.* (1989) reported a colonization of the collagen membrane by connective tissue cells with eventual degradation and replacement by new collagen. In the present study there was no distinguishable separation between collagen membrane and bone in most sites. A similar appearance was seen between membrane and cementum in one site. Similar to the multi-nucleated giant cell reaction seen with the PTFE membranes, one site treated with collagen membrane plus TGF-ß1 demonstrated multinucleated giant cells. No apparent clinical exposure of the membrane nor alteration in clinical healing was apparent. Although giant cells were present, the membrane was in close approximation with new bone and no difference in regeneration of the periodontium was seen compared to the other collagen membrane sites. The fact that the membranes were still intact at five weeks in this study agree with Pfeifer *et al.* (1989), illustrating that cross-

linked membranes have the capacity to remain intact for at least 5 weeks. Regeneration of cementum, a mixed orientation of periodontal ligament fibers and alveolar bone were present in non-titanium sites. Although there were no five week control sites, regeneration of the periodontium was present in the 20 week control sections. No conclusions can be made regarding the capacity of the membranes to encourage regeneration in this study. Overall, the collagen membrane used in this study was biocompatible in the rhesus monkey animal model.

Membranes were initially hypothesized to provide epithelial exclusion from the healing periodontium and to provide a space for the migration and population of the root surface with selective cell populations originating from the periodontal ligament and/or the alveolar bone. Aukhil and Iglhaut (1988) found no influence of filters by themselves on the mitosis of periodontal ligament cells. One aspect of this pilot study was to determine the influence of TGF-ß1 on the regeneration of the wounded periodontium. Ideally, substances could be either applied to the root surface and/or combined with membranes to increase the migration and mitosis of selective cells to repopulate the lost periodontium.

Polypeptide growth factors comprise a group of naturally occurring biologically active molecules that play a role in wound healing and immunoregulation. TGF-ß1 is a 25,000 molecular weight disulfide-linked dimer of two identical chains comprised of 112 amino acids (Sporn *et al.*, 1986, 1987). It has proven to be identical in various species including the human (Derynck *et al.*, 1985), monkey (Sharples *et al.*, 1987), bovine (Van Obberghen-Schilling *et al.*, 1987) and porcine (Derynck and Rhee 1987). Several types of cells produce TGF-ß1, including osteoblasts, fibroblasts, platelets and mesothelial cells (Gehron-Robey *et al.*, 1987, Gerwin *et al.*, 1987, Lawrence *et al.*, 1984). Most cell types have receptors for and bind TGF-ß1, which has been demonstrated to modulate the cell's activity (Cheifetz *et al.*, 1986, Wakefield *et al.*, 1988). This biologic modulator has a range of activities, being stimulatory for some cells and inhibitory for others. One

such effect is regulation of bone remodeling. TGF-ß1 has an inhibitory effect on osteoclast-like cells (Pfeilschifter *et al.*, 1987; Chenu *et al.*, 1988; Oreffo *et al.*, 1988, 1990; Shinar *et al.*, 1990) and a stimulatory effect on osteoblasts (Hock *et al.*, 1990). *In vitro* studies have demonstrated TGF-ß to induce a mitogenic response in periodontal ligament cells and to be a chemoattractant for periodontal ligament cells (Kowalski *et al.*, 1988; Cotugno *et al.*, 1988). Skin wound healing studies in pigs have demonstrated an increased effect on new connective tissue, collagen and angiogenesis by TGF-ß1, while epithelization was inhibited and inflammation was enhanced (Lynch *et al.*, 1988, 1989).

This pilot project sought to examine the effect of TGF-ß1 incorporated in a collagen membrane on the healing efficacy within the fenestration wound model. The concentration of TGF-ß1 utilized, 2.5 ug per square centimeter of membrane, was based on an *in vivo* dose response experiment demonstrating increased fibrous tissue deposition (Chu 1991). No differences were demonstrable between membrane sites with or without incorporation of TGF-ß1. This may have been due to the healing time evaluated and/or the healing capacity of the fenestration wound model. Potentially, the five week healing time may have been too long to demonstrate potential differences between collagen membranes with or without TGF-ß1. Alternatively, since the growth factor was incorporated in the membrane and due to the membrane stiffness which allowed it to bridge the fenestration, the growth factor may not have been available at the root surface. In the one site where an attempt was made to fill the defect with the membrane, it formed a physical barrier that may have inhibited or delayed bone formation. Alternative carrier systems, such as collagen gels, may prove more efficacious in delivering growth factors in critical wound healing sites. No adverse effects of the growth factor were demonstrated. In addition, shorter time periods need to be observed to compare the influence of growth factors on wound healing. Iglhaut *et al.* (1988) observed cell migration in fenestration wound sites in a similar monkey model

to be nearly complete by 21 days. Therefore, evaluation periods for growth factors would apparently be most efficacious if observed at shorter time intervals. Iglhaut *et al.* (1988) observed peak cell migration between 3 and 7 days. Alternatively, in addition to shorter evaluation times, larger defects could be utilized to evaluate the effect of various materials on cell chemotaxis.

Branemark (1977) proposed the term osseointegration to describe a bone to titanium implant interface. The latter is a light microscopic finding where bone is in direct contact with the implant surface with no intervening fibrous tissue. One of the parameters evaluated in this pilot project was formation of cementum adjacent to titanium surfaces. Rylander and Lundgren (1988) utilized similar methods and materials in a dog animal model, but no cementum formation occurred on the titanium surface. No previously reported studies utilizing this wound model have examined this parameter. Cementum formation on the titanium screw surfaces in this study demonstrated a direct contact between new cementum and titanium surface at the light microscopic level, thus producing a junction which may be identified as cementointegration. The twenty week evaluation period proved to be adequate for the observation of cementum formation on the titanium surface. The five week period was apparently sufficient for cementum formation on adjacent denuded dentin but not on the titanium surface. A recent study has also reported cementum formation on an adjacent titanium surface in a non-submerged wound model (Buser *et al.*, 1990).

Rylander *et al.* (1990) placed intra-alveolar titanium root-analogues in extraction sites and demonstrated increased area of osseointegration with increased time; however, there were no signs of cementum formation or inserting collagen fibers on the implant surface. The compilation of results from this pilot project and those of Buser *et al.* (1990) and Rylander *et al.* (1990) suggest that either a critical mass of adjacent periodontium is necessary to populate such a titanium surface or that residual periodontal tissue remaining in an extraction site could potentially undergo degradation

similar to that seen when an exfoliated tooth is replanted and subsequently undergoes resorptive ankylosis.

Titanium cones have been utilized as retrograde restorations in apicoectomized teeth since 1981 with an apparent high degree of clinical success (Trippler *et al.*, 1987, Cordes *et al.*, 1987). Unfortunately, no histology has been reported. It would appear that this treatment would potentially produce similar findings with regeneration of the periodontium over the titanium retrograde restoration. If the findings in this pilot study and that of Buser *et al.* (1990) can be consistently reproduced, there are several additional applications where treatment may be improved. For example, root fenestrations and vertical fractures may be treatable with titanium restorations. In addition, furcation invasions may be occluded with a custom titanium implant creating a combination of osseointegration adjacent to the bone interface and cementointegration adjacent to the dental interface. Indeed it may be possible to graft vertical osseous defects, such as interdental craters, with a titanium graft if the adjacent vascular supply is adequate to maintain the overlying soft tissues. Horizontal dental fractures apical to the alveolar crest may be restorable with a titanium casting replacing the lost tooth structure. Many of these treatments would maintain proprioception, unlike that found in currently used osseointegrated dental implants.

During dentinogenesis it is hypothesized that dental follicle cells differentiate into cementoblasts when they come into contact with dentin following disruption of Hertwig's epithelial root sheath (Ten Cate 1980). Variations obviously exist as cementum overlaps the apical extension of the enamel surface in approximately 60% of teeth. Histologically, the enamel epithelium degenerates at its apical extent, allowing connective tissue to contact the enamel surface (Bhaskar, 1980). During regeneration of the lost periodontium, progenitor cells apparently arise from adjacent periodontal ligament and/or bone to form new cementum and periodontal ligament (Melcher 1970, Gould *et al.*, 1980, Karring *et al.*, 1980, Nielsen *et al.*, 1980, Karring *et al.*, 1985, Nyman

et al., 1982, Iglhaut *et al.*, 1988). Similar to the differentiation hypothesized to occur during dentinogenesis, periodontal ligament progenitor cells are believed to differentiate into cementoblasts when they come into contact with dentin during regeneration (Aukhil *et al.*, 1986). The results of this study contradict the findings of Aukhil *et al.* (1986) who suggested that "...differentiation (of cementoblasts) can be prevented in interposing a physical barrier between the progenitor cells and the root dentin." Contact with dentin is apparently not necessary as new cementum was found to form on a titanium surface in a similar fenestration wound model as that used by Aukhil *et al.* (1986). These findings support those of Buser *et al.* (1990) which similarly reported cementum formation on titanium implants placed adjacent to retained root tips in a non-submerged wound model. In addition, new cementum formation on preexisting cementum was seen in this study as well as in previous fenestration studies (Beube 1947, Register 1973). The compilation of these findings suggest that dentin may not necessarily possess an inherent property necessary to induce cementogenesis other than that of biocompatibility.

VI. SUMMARY

The following findings of this pilot project have further defined the fenestration wound model in the Rhesus monkey animal model.

1. This fenestration wound model has a high propensity for healing, which suggests it may serve as a valuable model for the assessment of wound healing events.
2. The observation of healing events in the absence of disease provides a predictable model for evaluating optimal effects of various periodontal regenerative treatments.
3. Generation of periodontium is possible on an artificial surface demonstrated by cementum formation on a titanium surface and formation of functionally oriented collagen fibers inserted in the new cementum surface.
4. Regeneration of the periodontium is complete in this model by 20 weeks.
5. Regeneration of the periodontium may occur in this fenestration wound model with and without exclusion membranes.
6. A biodegradable collagen membrane is biocompatible in the Rhesus monkey animal model.
7. No apparent histological differences exist in a 5 week observation of wound healing when collagen membranes with and without TGF-ß1 are placed over the fenestration wounds.
8. A foreign body giant cell reaction may occur adjacent to both PTFE and collagen membranes without any apparent adverse effect on healing.

These findings and lack of dissimilarities between treatment modalities may be related to the size of defect and evaluation times used in this study. Further limitations of this model are related to technical difficulties related to the treatment modalities utilized, post surgical flap recession and pulpal exposure.

Future studies using the fenestration wound animal model system should be directed toward evaluating: (1) if a critical size periodontal fenestration defect exists in this animal model; (2) shorter evaluation times utilizing various carrier systems including

collagen gels and additional growth factors; (3) the sequence and quantification of various contributing cells in periodontal wound healing upon the availability of cell markers such as monoclonal antibodies; (4) titanium as a retrograde restoration, root fracture restoration and osseous defect implant graft; (5) the critical mass of periodontium necessary to achieve regeneration on an artificial surface to determine if an implant may be accomplished with a normal periodontium; (6) other implant surfaces to determine if similar regeneration is possible.

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